

Supplementary information

Supplementary table S1. Assembly statistics.

Species	Tissue	Number of PE reads	Number of raw sequencing bases	Number of contigs	Number of contigs >300bp	Contig N50 (bp)	Max contig (bp)
Eco	VG	110,032,016	22,173,182,778	117,125	81,798	2,623	30,131
	SK	14,166,420	2,833,284,000	77,402	50,860	1,725	30,610
	SCG	27,206,987	5,441,397,400	138,852	87,389	2,444	36,612
	Liver	7,095,517	1,433,294,434	31,205	18,169	950	9,939
	Brain	31,934,884	6,450,846,568	195,958	134,236	3,552	20,155
	Ovary	18,155,364	3,667,383,528	81,682	52,264	2,023	16,376
	KID	41,148,101	8,311,916,402	120,728	76,660	2,044	12,930
Epy	VG	57,398,601	11,594,517,402	127,519	86,953	2,898	29,658
Pgu	SAL	25,655,661	5,131,132,200	64,595	43,565	1,916	17,102
Oae	SAL	24,959,242	4,991,848,400	65,393	42,558	1,780	17,524
Pre	SAL	28,035,045	5,607,009,000	73,492	48,727	2,265	33,655
Ema	SAL	29,882,110	5,976,422,000	111,345	73,027	2,439	24,285
Oha	VG	15,166,590*	834,162,450	6,123	2,925	424	4,585
	AG	11,209,677*	616,532,235	9,046	4,113	377	3,740
	PT	17,858,289*	910,772,739	8,877	4,135	413	5,733

Species abbreviations:

Eco, painted saw-scaled viper (*Echis coloratus*); Epy, Egyptian saw-scaled viper (*Echis pyramidum*); Pgu, corn snake (*Pantherophis guttatus*); Oae, rough green snake (*Opheodrys aestivus*); Pre, royal python (*Python regius*); Ema, leopard gecko (*Eublepharis macularius*); Oha, king cobra (*Ophiophagus hannah*).

Tissue abbreviations:

VG, venom gland; SK, skin; KID, kidney; SAL, salivary gland; AG, accessory gland; PT, pooled tissue.

*These values are for single-end sequencing reads.

Supplementary table S2. Predicted open reading frame statistics and details of BLAST-based gene ontology (GO) annotation of venom and salivary gland transcriptomes.

	Total contigs	Number of ORFs	Mean ORF length (nt)	Max ORF length (nt)	Number of ORFs with signal peptide	Number of ORFs with signal peptide and BLAST result	Number of ORFs with GO annotation
Eco	56,805	56,761	459	13,642	2,655 (4.68%)	1,341 (2.36%)	896 (1.58%)
Epy	86,953	86,908	699	28,315	4,574 (5.26%)	2,590 (2.98%)	2,020 (2.32%)
Pgu	43,565	43,534	548	12,139	2,197 (5.05%)	1,252 (2.88%)	909 (2.09%)
Oae	42,558	42,534	502	14,314	1,908 (4.49%)	916 (2.15%)	702 (1.65%)
Pre	48,727	48,690	544	33,010	2,247 (4.61%)	1,097 (2.25%)	868 (1.78%)
Ema	73,027	72,980	540	24,064	3,702 (5.07%)	1,856 (2.54%)	1,436 (1.97%)
Oha VG	6,123	6,102	233	2,896	227 (3.72%)	102 (1.67%)	82 (1.34%)
Oha AG	9,046	9,023	234	3,454	353 (3.91%)	N/A	N/A
Mfu	2,066	2,066	1,233	2,066	220 (10.64%)	190 (9.19%)	165 (7.98%)
Cad	12,694	12,694	874	11,752	771 (6.07%)	538 (4.23%)	411 (3.23%)

Species abbreviations:

Eco, painted saw-scaled viper (*Echis coloratus*); Epy, Egyptian saw-scaled viper (*Echis pyramidum*); Pgu, corn snake (*Pantherophis guttatus*); Oae, rough green snake (*Opheodrys aestivus*); Pre, royal python (*Python regius*); Ema, leopard gecko (*Eublepharis macularius*); Oha, king cobra (*Ophiophagus hannah*); Mfu, Eastern coral snake (*Micrurus fulvius*); Cad, Eastern diamondback rattlesnake (*Crotalus adamanteus*)

Supplementary table S3. Predicted open reading frame statistics and details of BLAST-based gene ontology (GO) annotation of painted saw-scaled viper (*Echis coloratus*) tissue transcriptomes.

Tissue abbreviations:

	Total contigs	Number of ORFs	Mean ORF length (nt)	Max ORF length (nt)	Number of ORFs with signal peptide	Number of ORFs with signal peptide and BLAST result	Number of ORFs with GO annotation
VG	44,470	44,445	478	7,705	2,146 (4.83%)	1,367 (3.08%)	984 (2.21%)
SCG	67,857	67,813	557	23,131	3,182 (4.69%)	2,083 (3.07%)	1,581 (2.33%)
SK	44,805	44,760	479	28,480	1,994 (4.45%)	1,299 (2.90%)	972 (2.17%)
Brain	78,074	78,022	615	13,945	3,878 (4.97%)	2,122 (2.72%)	1,694 (2.17%)
Kidney	51,969	51,942	456	13,990	2,257 (4.34%)	1,070 (2.06%)	803 (1.55%)
Ovary	52,264	52,227	584	13,765	2,643 (5.06%)	1,470 (2.81%)	1,159 (2.22%)
Liver	18,169	18,159	346	6,775	881 (4.85%)	450 (2.48%)	368 (2.03%)

VG, venom gland; SCG, scent gland; SK, skin.

Supplementary table S4. Predicted open reading frame statistics and details of BLAST-based gene ontology (GO) annotation of painted saw-scaled viper (*Echis coloratus*) venom gland transcriptomes taken at different timepoints following milking.

	Total contigs	Number of ORFs	Mean ORF length (nt)	Max ORF length (nt)	Number of ORFs with signal peptide	Number of ORFs with signal peptide and BLAST result	Number of ORFs with GO annotation
Eco 8	53,786	53,744	631	15,070	2,629 (4.89%)	1,662 (3.09%)	1,269 (2.36%)
Eco 6	44,470	44,445	478	7,705	2,146 (4.83%)	1,347 (3.03%)	984 (2.21%)
Eco 7	51,505	51,472	624	20,434	2,796 (5.43%)	1,894 (3.68%)	1,412 (2.74%)
Eco 215	48,321	48,284	429	6,622	2,387 (4.94%)	1,483 (3.07%)	1,009 (2.09%)

Supplementary table S5. Transcriptome metrics and details of BLAST-based gene ontology (GO) annotation of venom gland sequences which are unique to a specific timepoint/sample post-venom extraction.

Venom gland	Time post-milking	Total number of transcripts expressed	Number of unique transcripts in sample	Number of unique transcripts with BLAST result	Number of unique transcripts with GO annotation
Eco 8	16 hr	28,448	5,082	2,203	1,496
Eco 7	24 hr	24,197	1,707	931	641
Eco 6	24 hr	37,834	7,325	2,701	1,727
Eco 215	48 hr	42,662	12,535	3,885	2,355

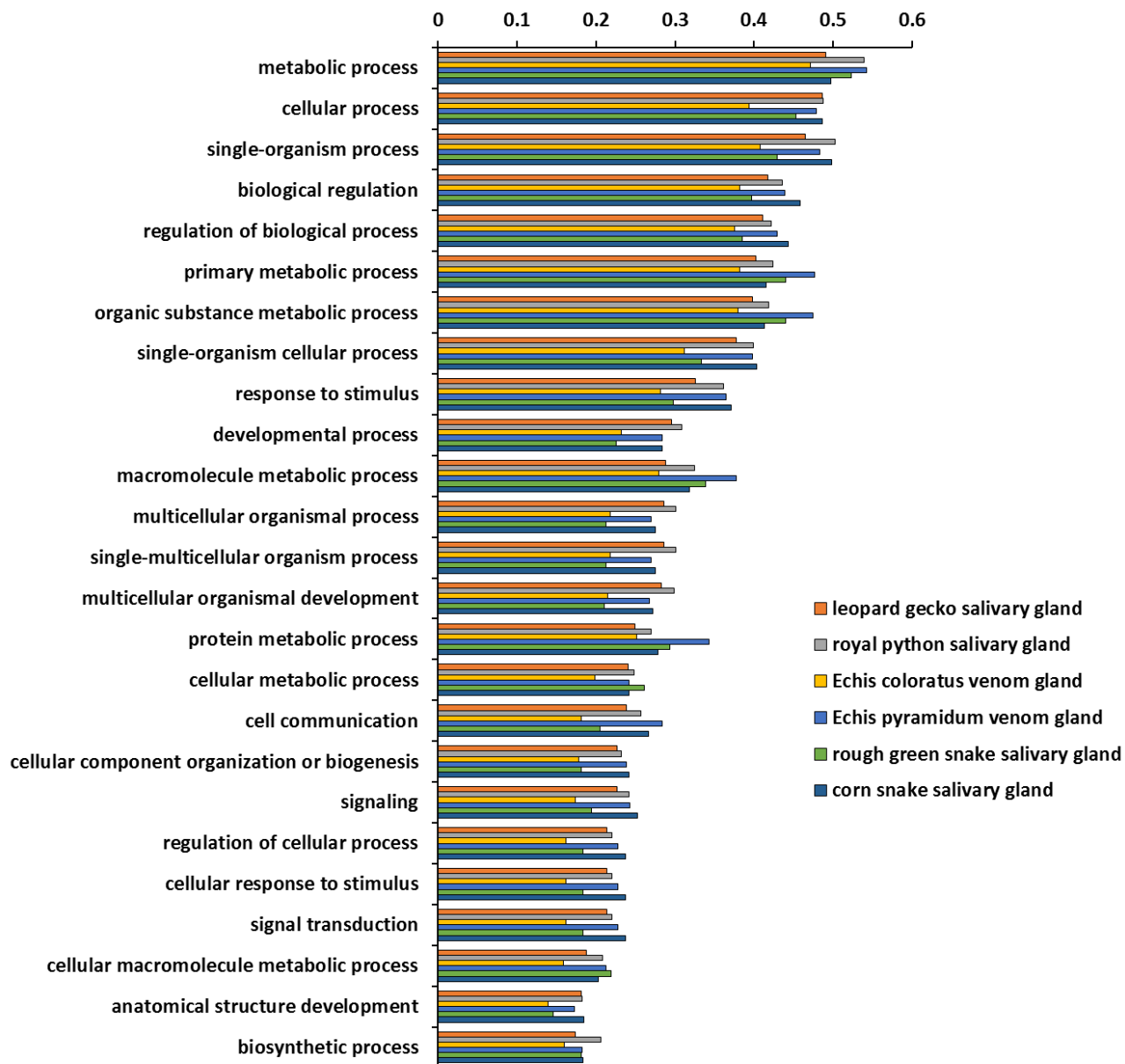
Supplementary table S6. Number of shared expressed transcripts between the venom gland and other body tissues of the painted saw-scaled viper, *Echis coloratus*.

Tissues	Number of shared transcripts
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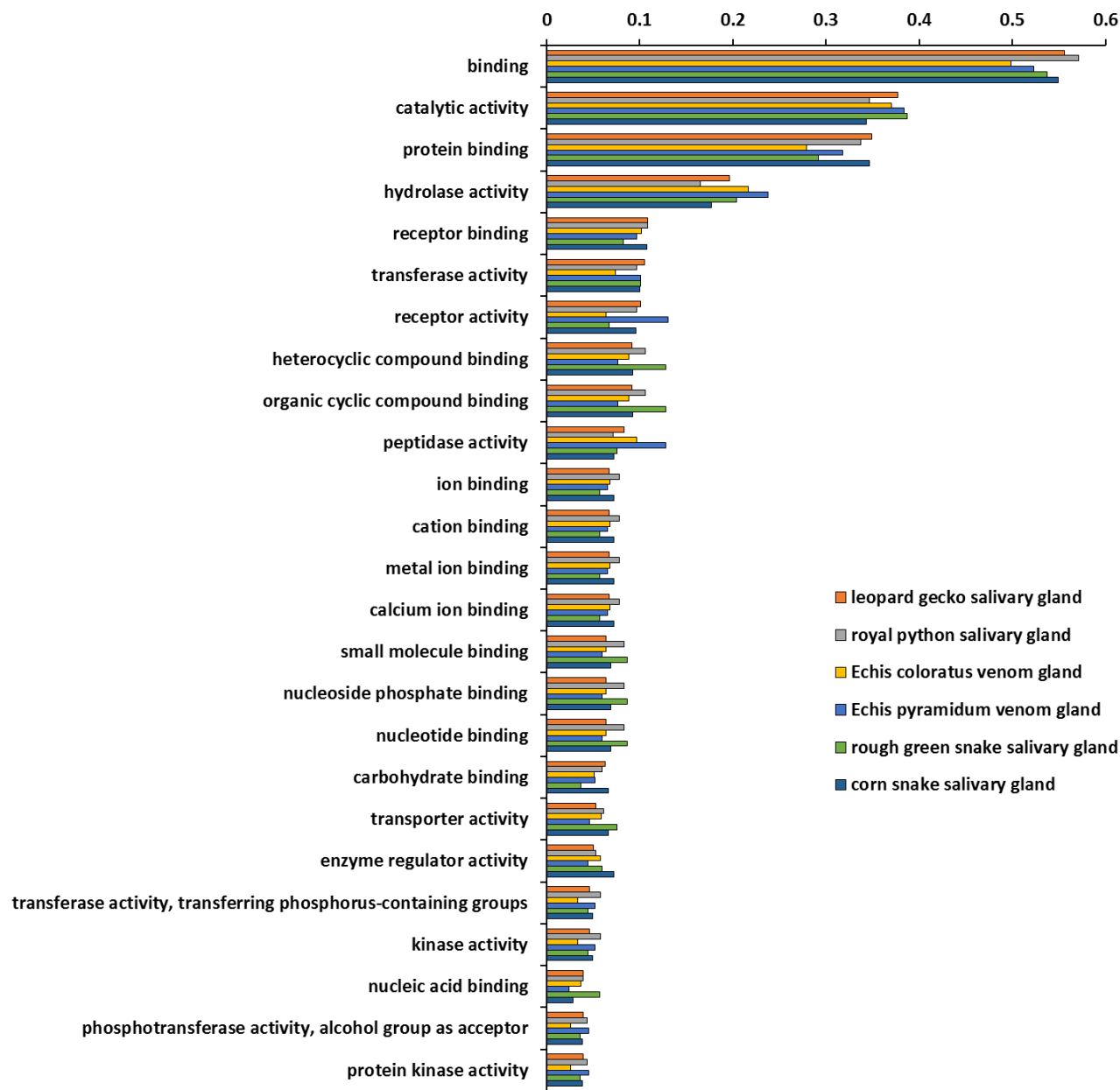
Venom gland + Scent gland	26,181
Venom gland + Brain	25,172
Venom gland + Skin	24,427
Venom gland + Ovary	23,255
Venom gland + Kidney	22,854
Venom gland + Liver	15,433

Supplementary table S7. Assembly metrics for the genome of the painted saw-scaled viper, *Echis coloratus*

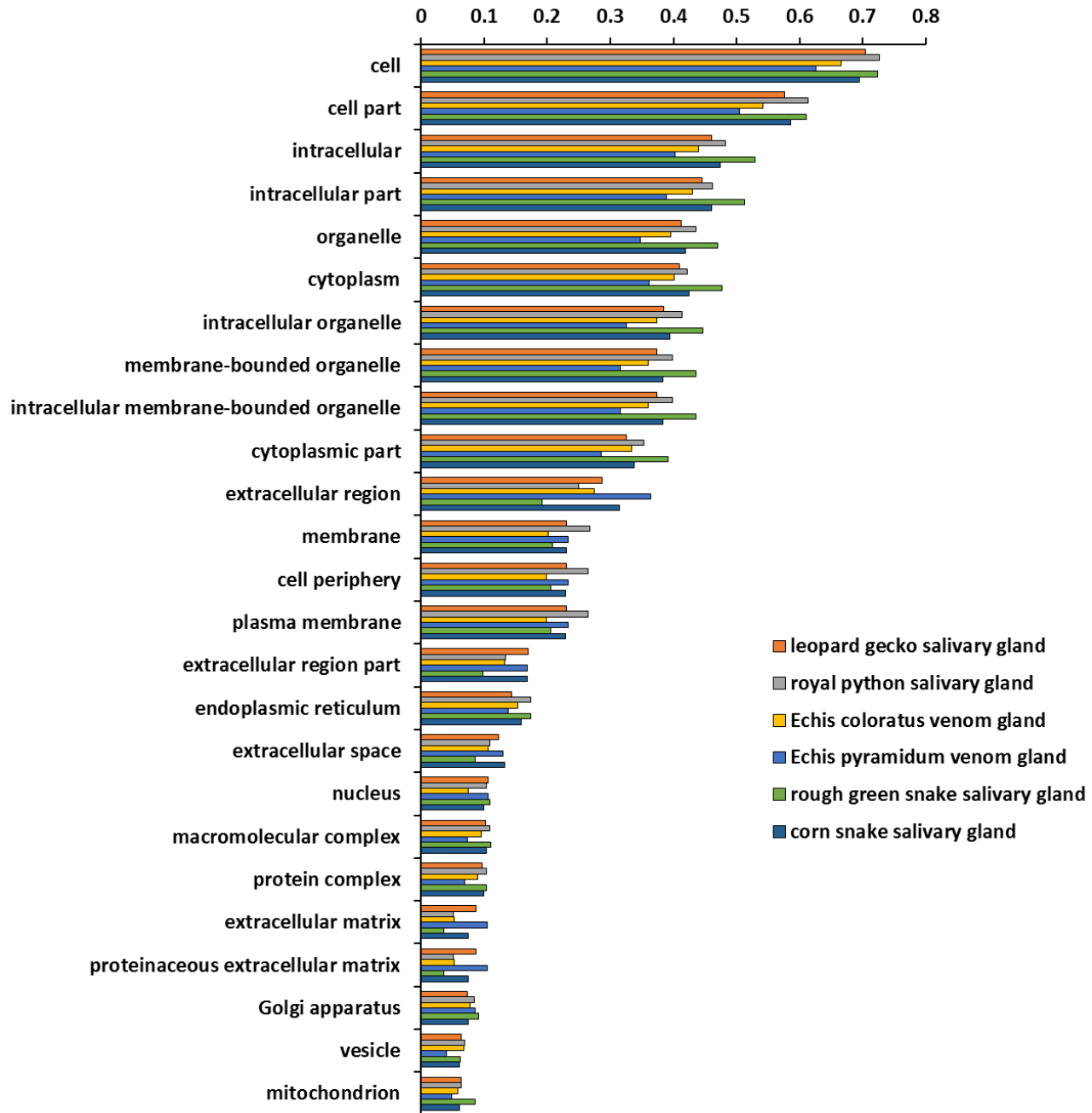
Total PE reads	Raw sequencing bases	Total contigs	Total length of assembly (bp)	Max contig length	Contig N50	Number of scaffolds	Max scaffold length	Scaffold N50
579,767,826	58,202,653,144	4,973,413	1,717,108,641	63,379	3,857	4,790,800	84,548	5,576



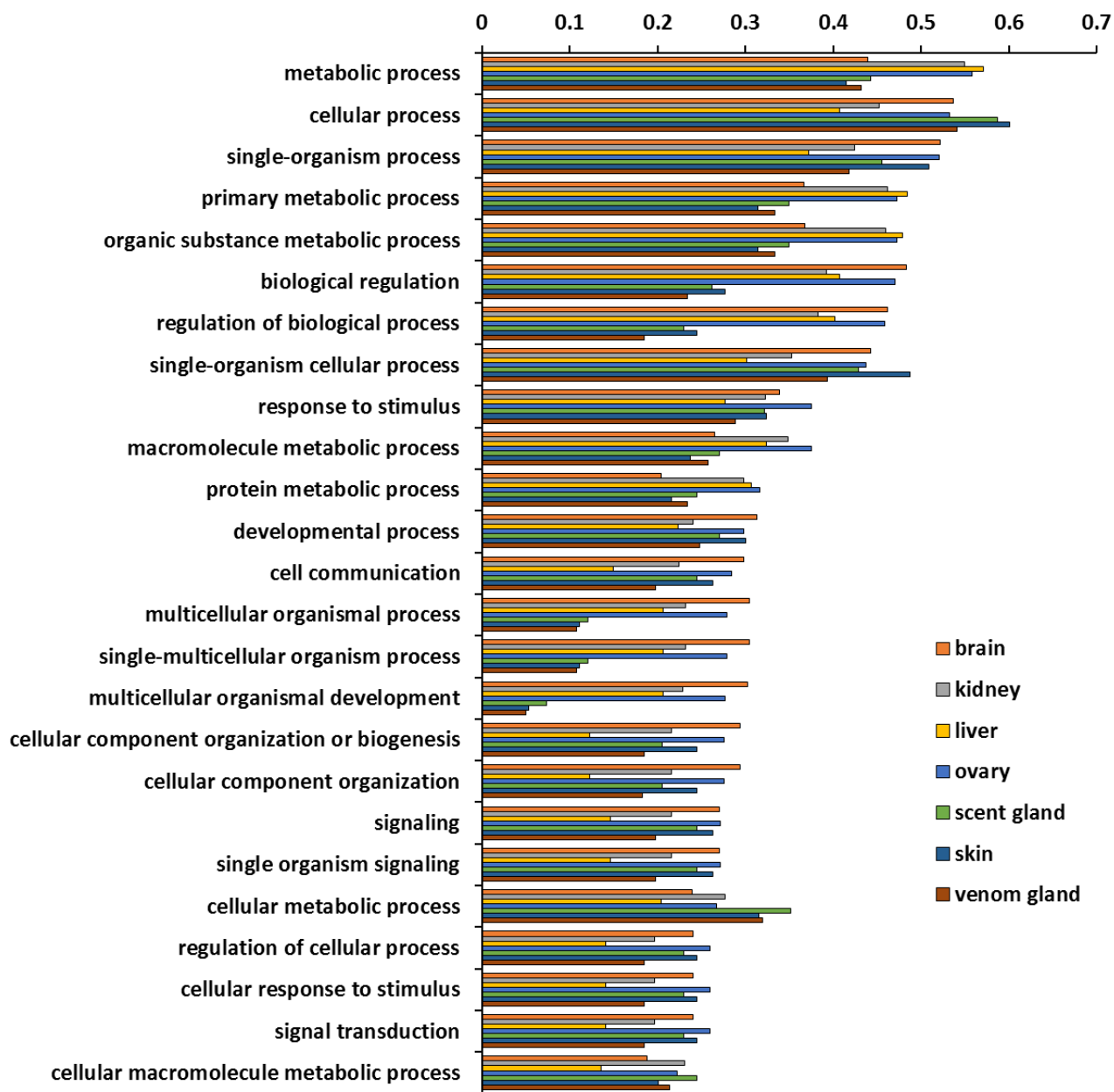
Supplementary figure S1. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Biological Process' terms for painted and Egyptian saw-scaled viper (*Echis coloratus* and *Echis pyramidum*) venom glands and corn snake (*Pantherophis guttatus*), rough green snake (*Opheodrys aestivus*), royal python (*Python regius*) and leopard gecko (*Eublepharis macularius*) salivary glands.



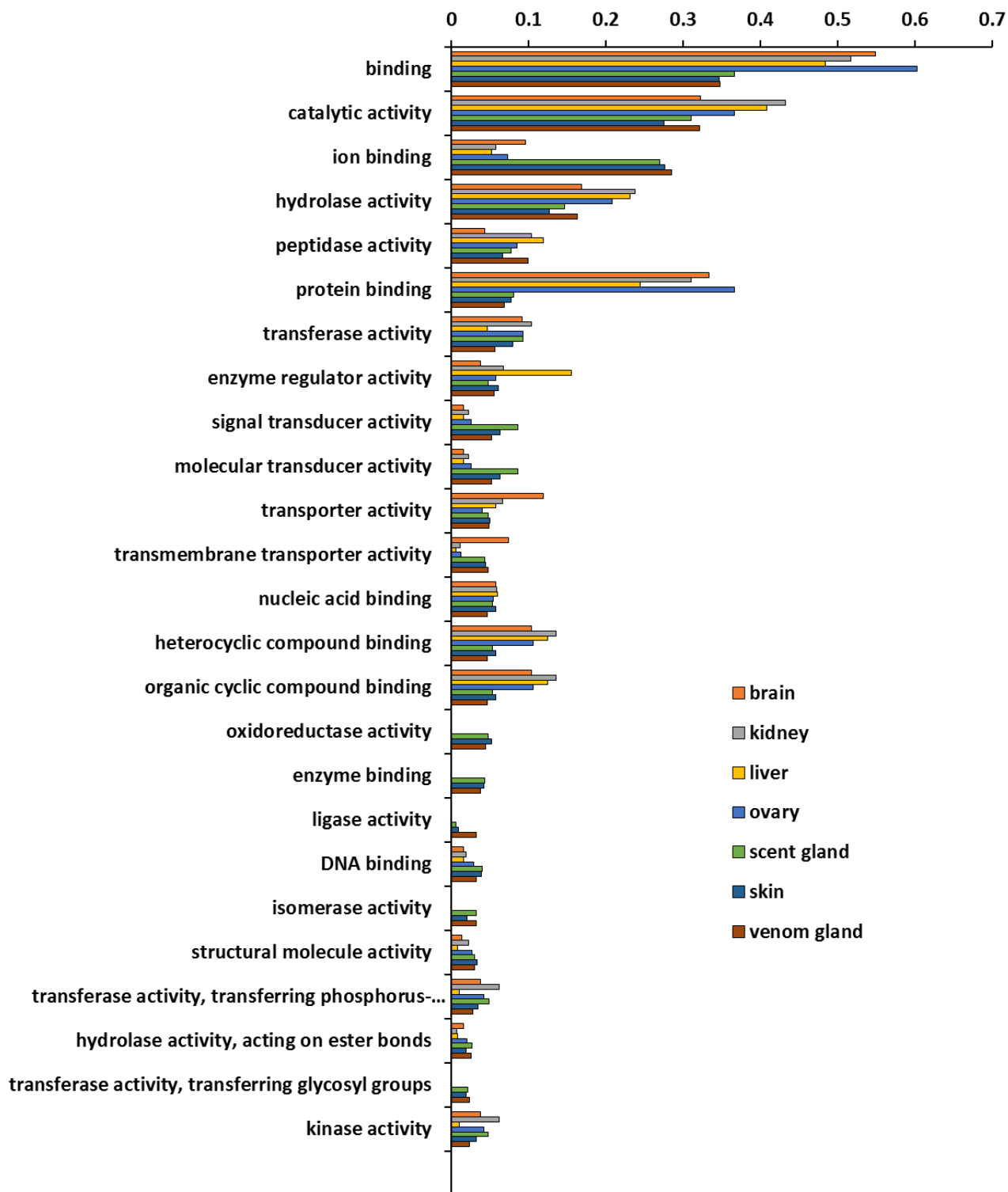
Supplementary figure S2. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Molecular function' terms for painted and Egyptian saw-scaled viper (*Echis coloratus* and *Echis pyramidum*) venom glands and corn snake (*Pantherophis guttatus*), rough green snake (*Opheodrys aestivus*), royal python (*Python regius*) and leopard gecko (*Eublepharis macularius*) salivary glands.



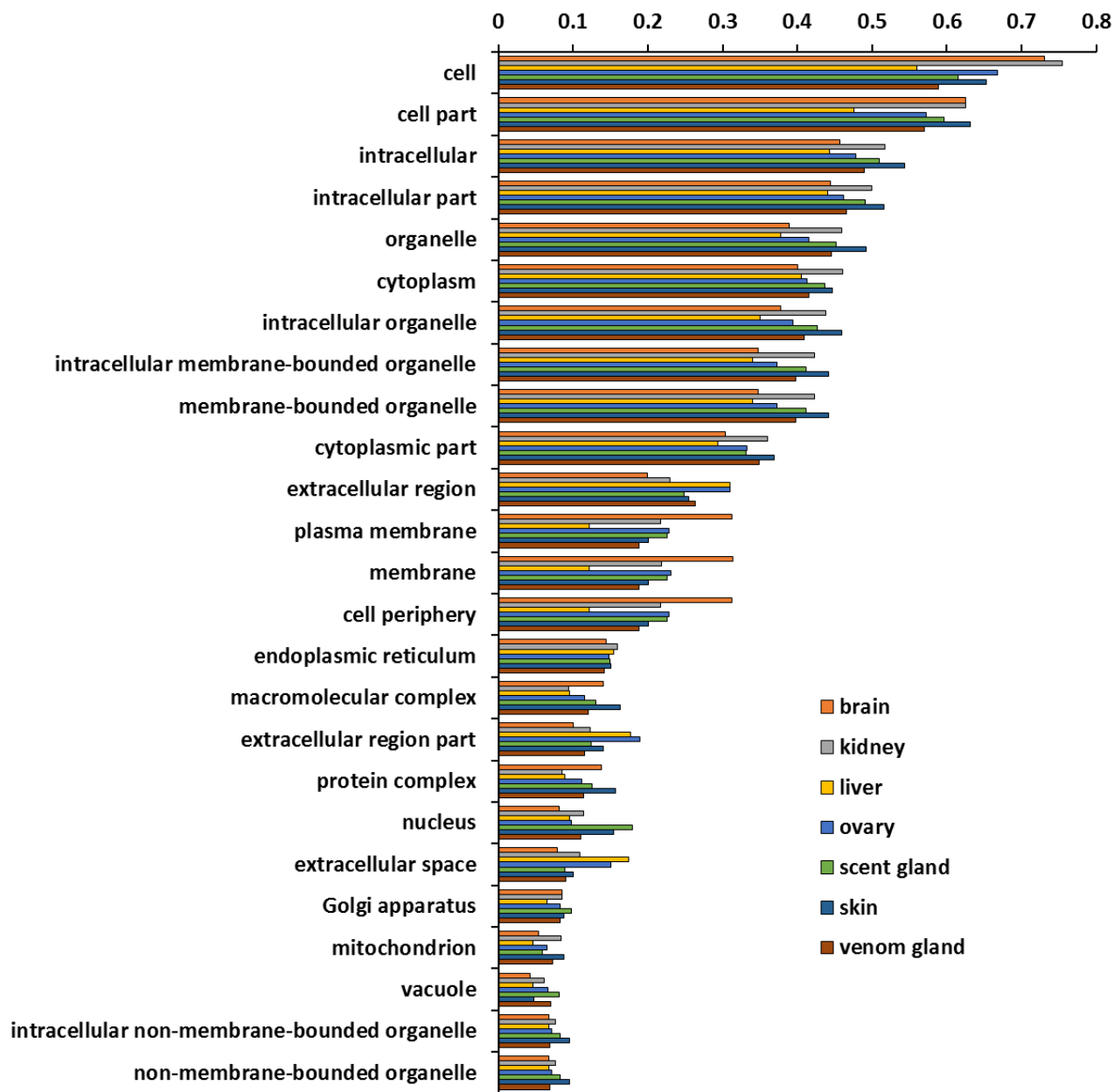
Supplementary figure S3. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim ‘Cellular component’ terms for painted and Egyptian saw-scaled viper (*Echis coloratus* and *Echis pyramidum*) venom glands and corn snake (*Pantherophis guttatus*), rough green snake (*Opheodrys aestivus*), royal python (*Python regius*) and leopard gecko (*Eublepharis macularius*) salivary glands.



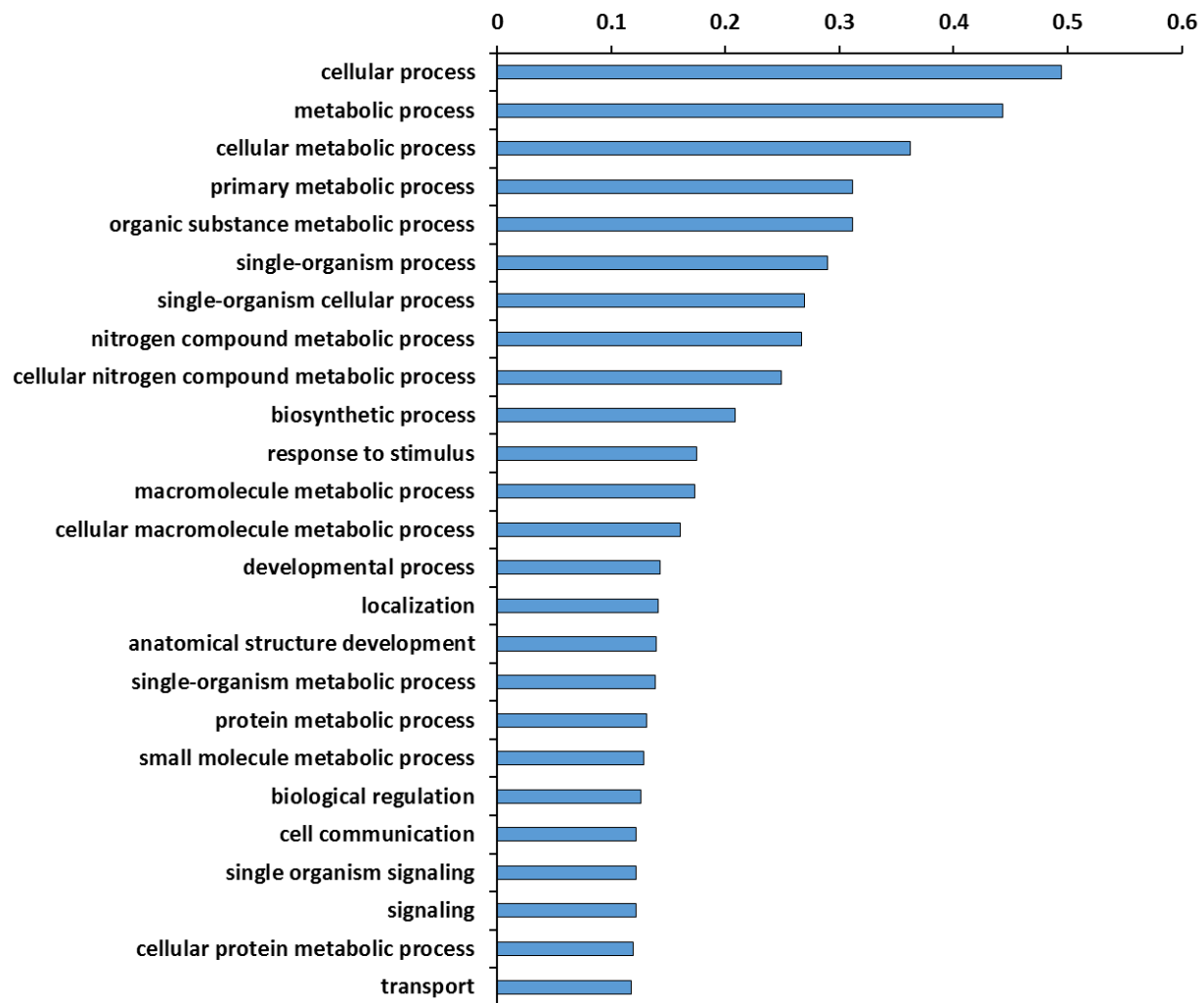
Supplementary figure S4. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Biological process' terms for painted saw-scaled viper (*Echis coloratus*) tissues.



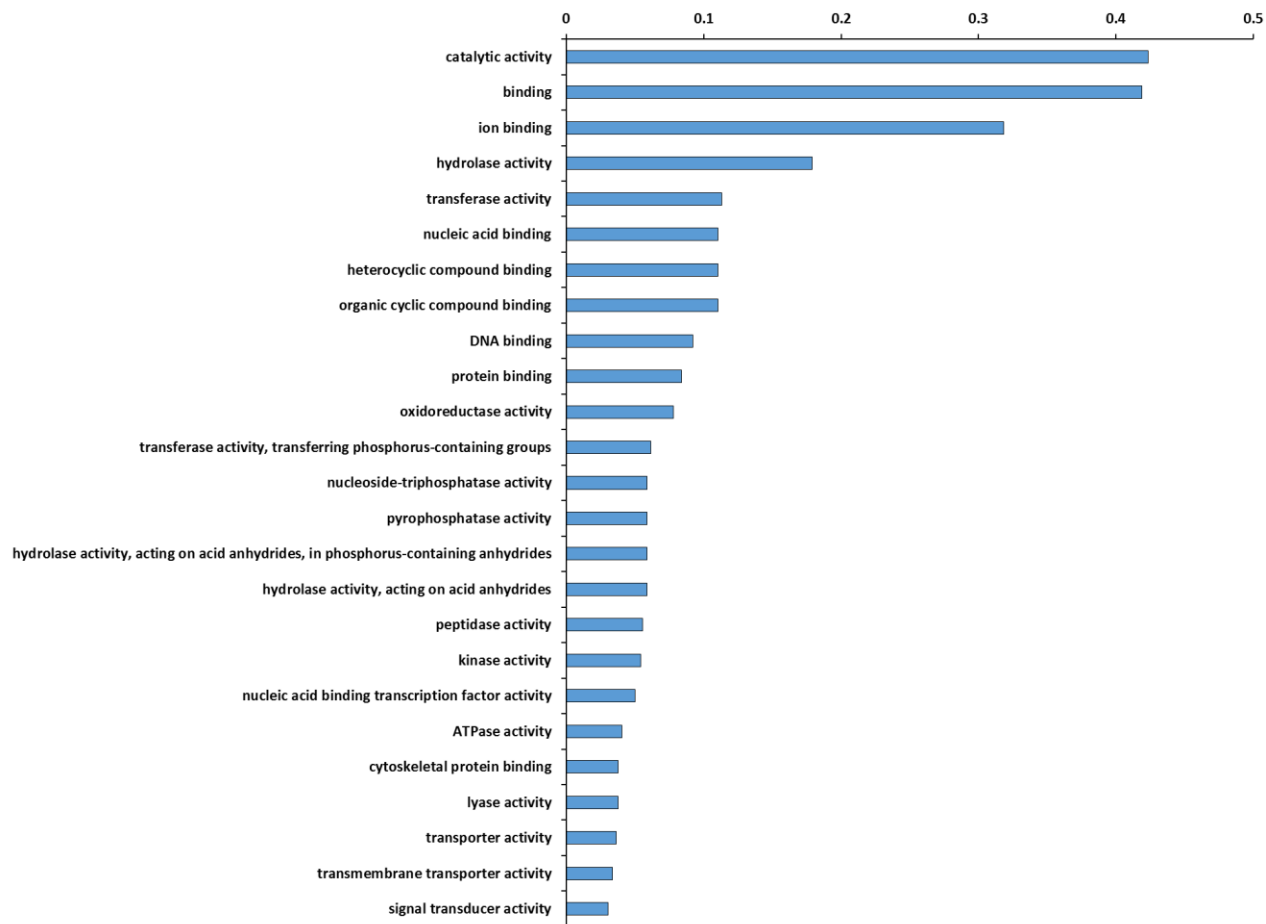
Supplementary figure S5. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Molecular function' terms for painted saw-scaled viper (*Echis coloratus*) tissues.



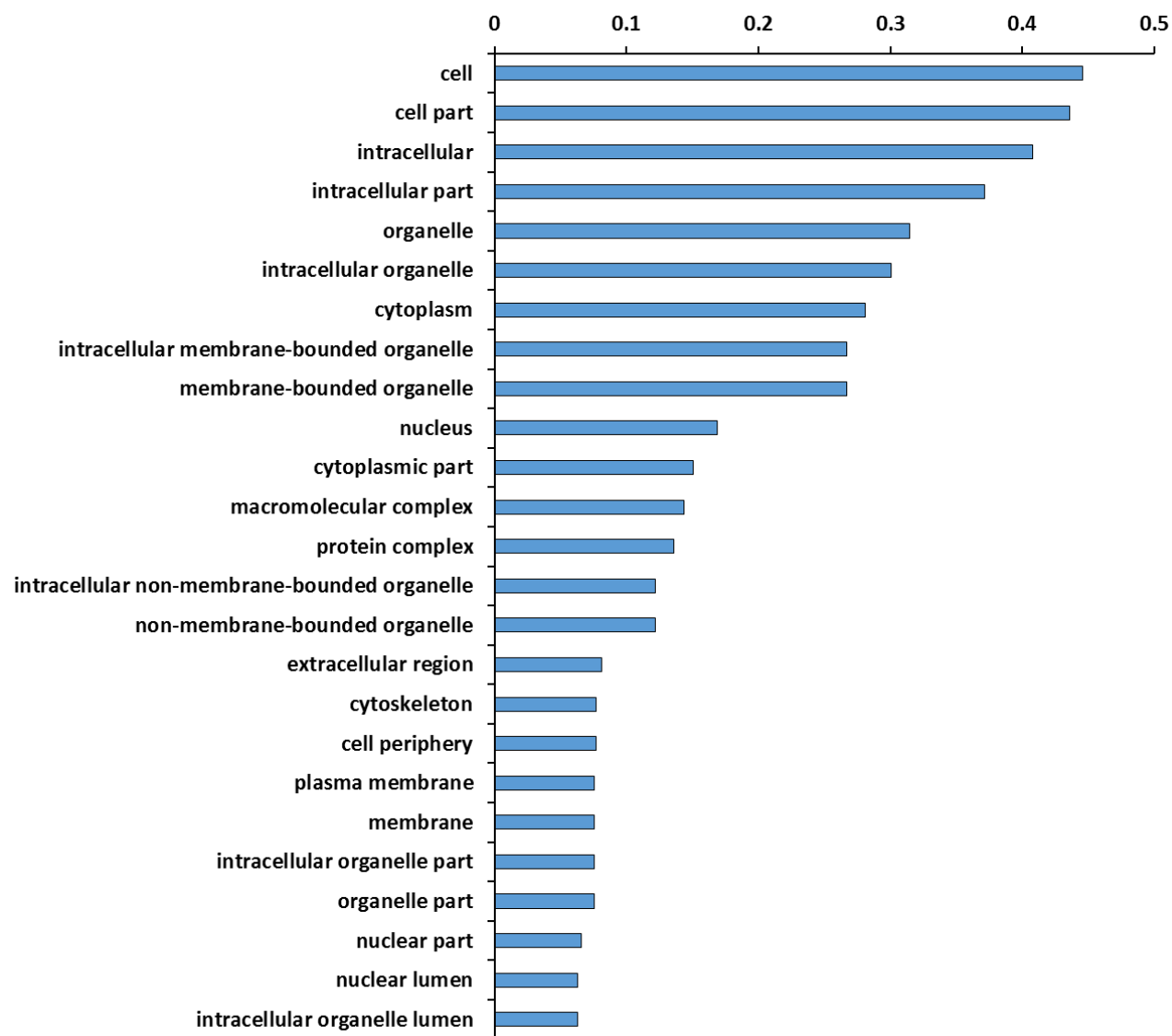
Supplementary figure S6. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Cellular component' terms for painted saw-scaled viper (*Echis coloratus*) tissues.



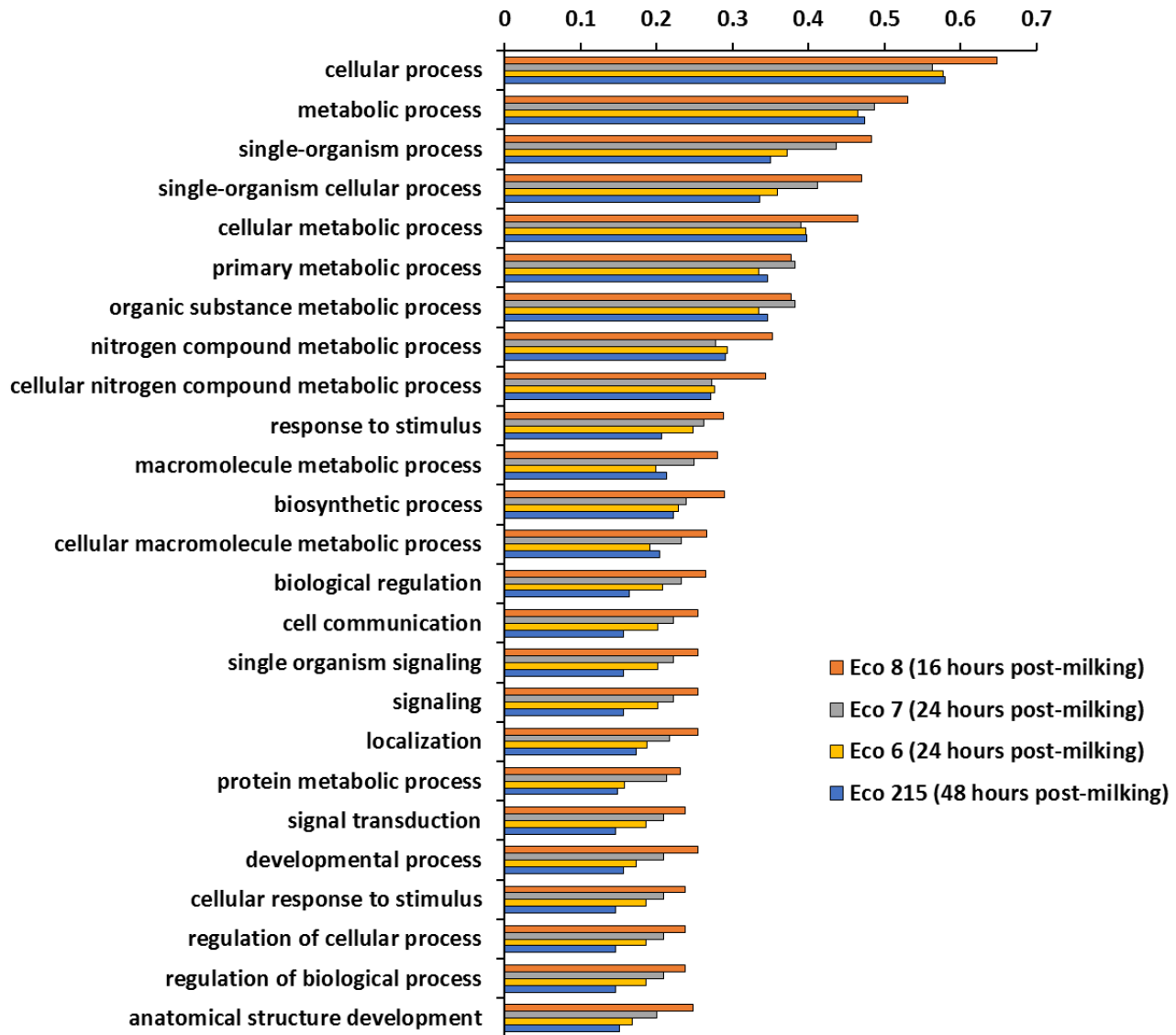
Supplementary figure S7. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Biological process' terms for expressed transcripts which are unique to the painted saw-scaled viper (*Echis coloratus*) venom gland compared to the remaining 6 body tissues.



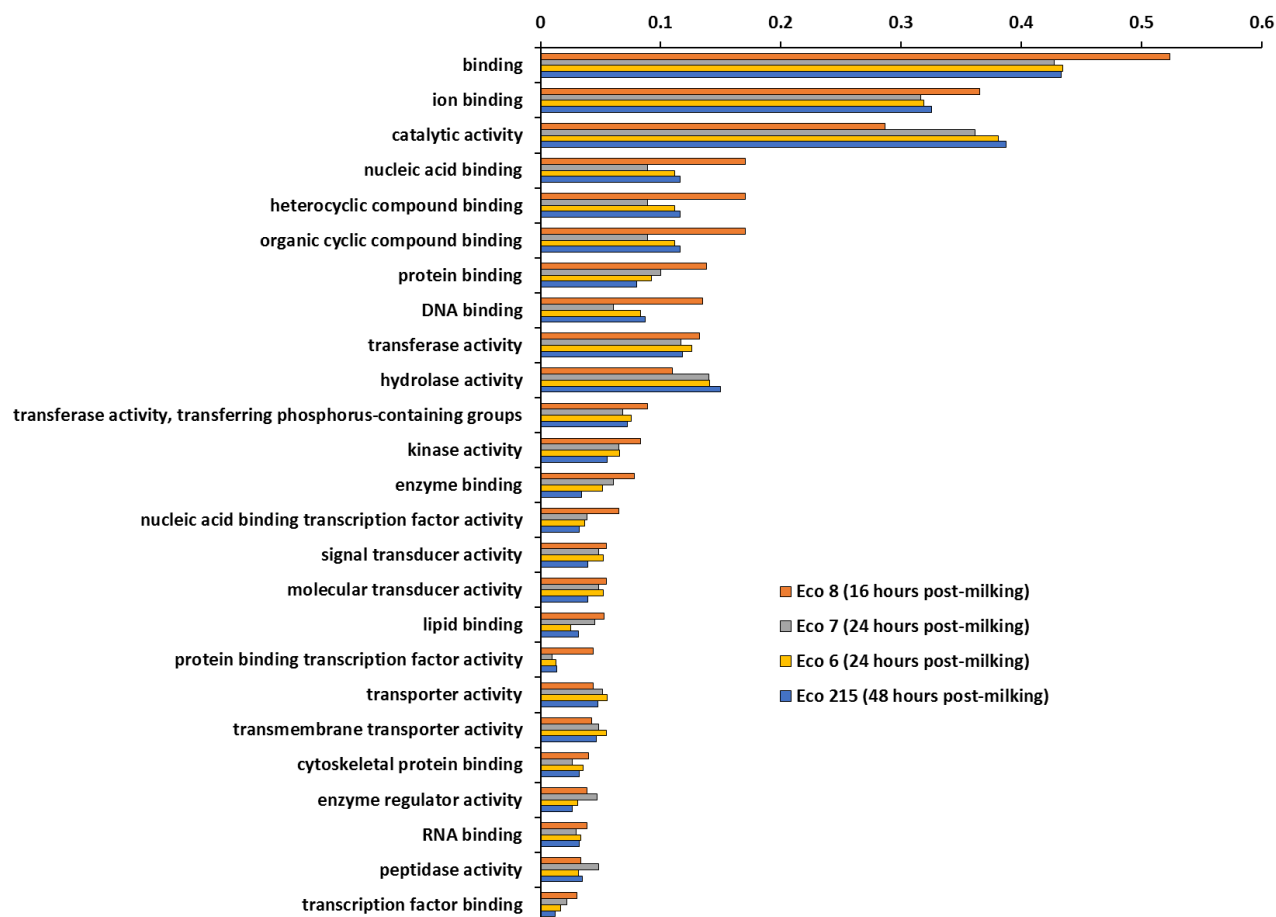
Supplementary figure S8. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Molecular function' terms for expressed transcripts which are unique to the painted saw-scaled viper (*Echis coloratus*) venom gland compared to the remaining 6 body tissues.



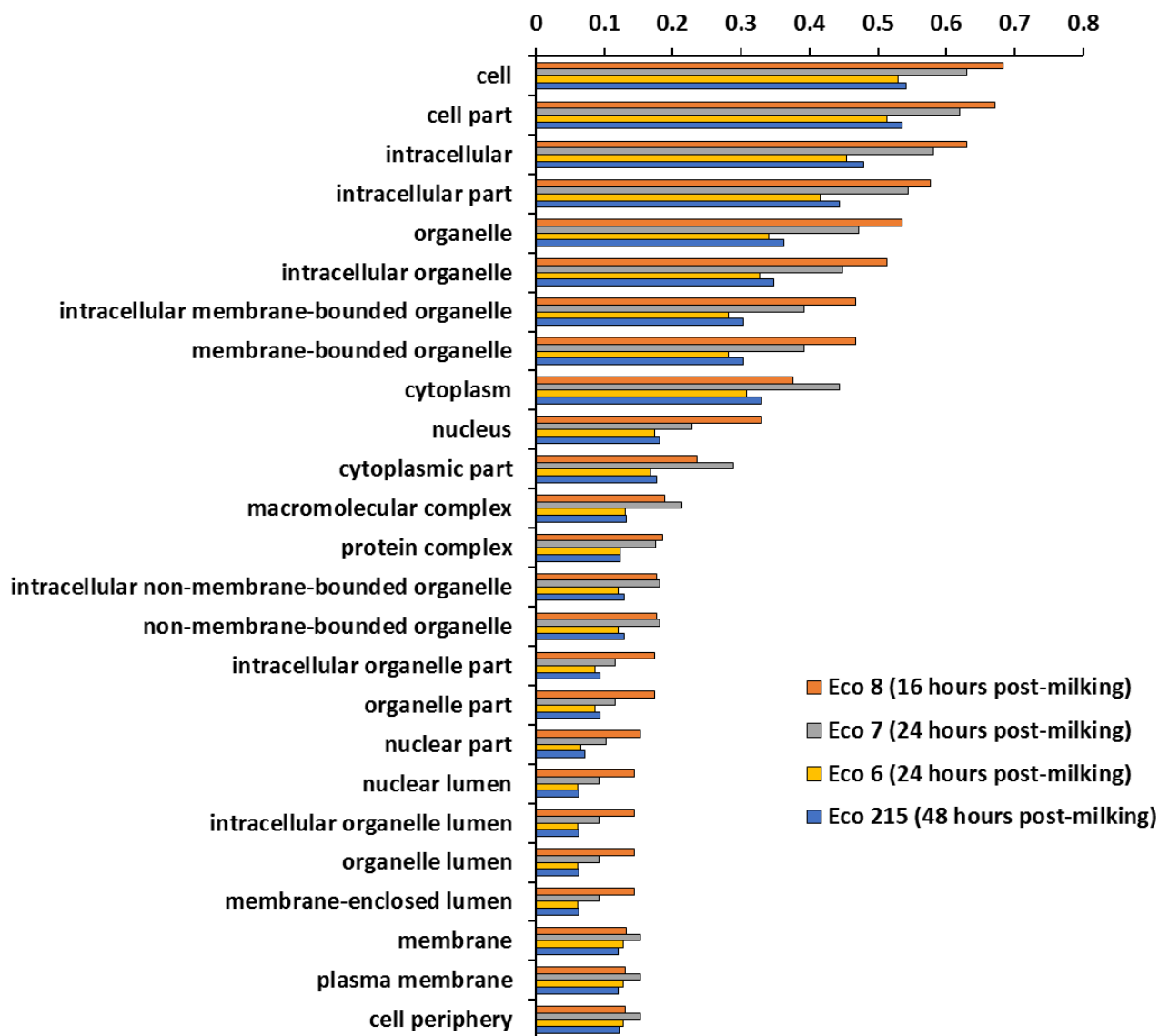
Supplementary figure S9. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim ‘Cellular component’ terms for expressed transcripts which are unique to the painted saw-scaled viper (*Echis coloratus*) venom gland compared to the remaining 6 body tissues.



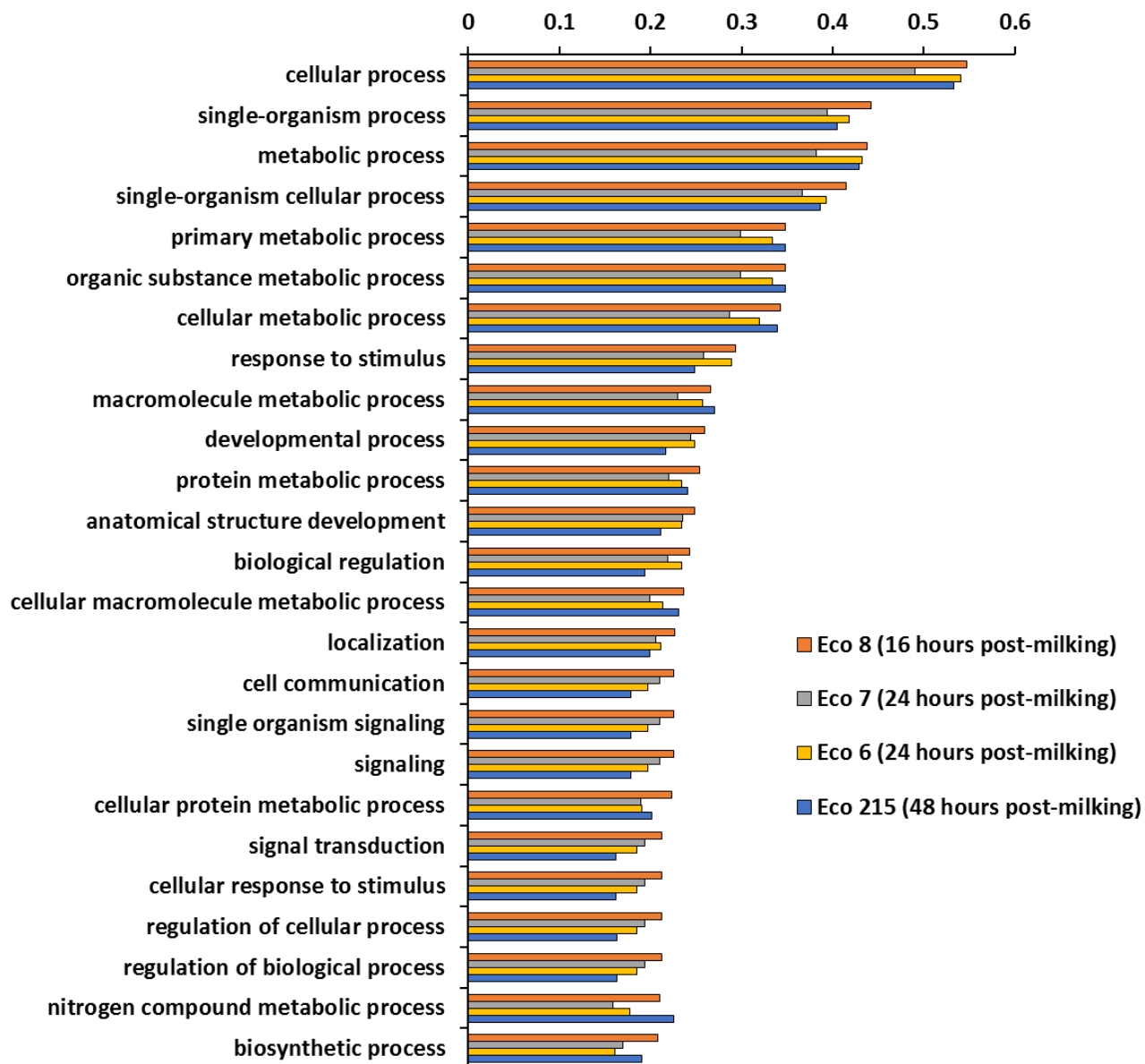
Supplementary figure S10. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Biological process' terms for transcripts which are unique to each individual timepoint following milking in the venom gland secretome of the painted saw-scaled viper (*Echis coloratus*).



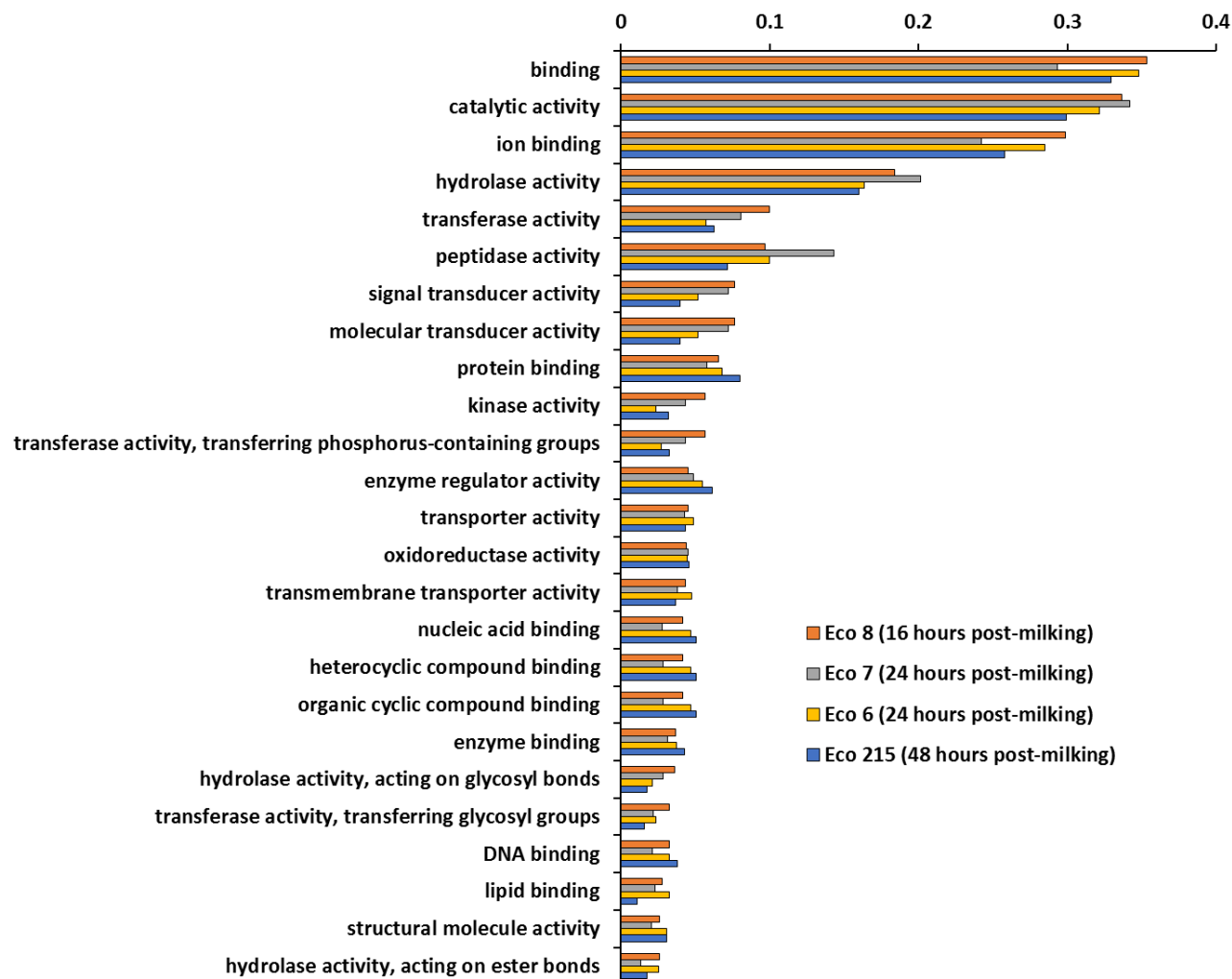
Supplementary figure S11. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Molecular function' terms for transcripts which are unique to each individual timepoint following milking in the venom gland secretome of the painted saw-scaled viper (*Echis coloratus*).



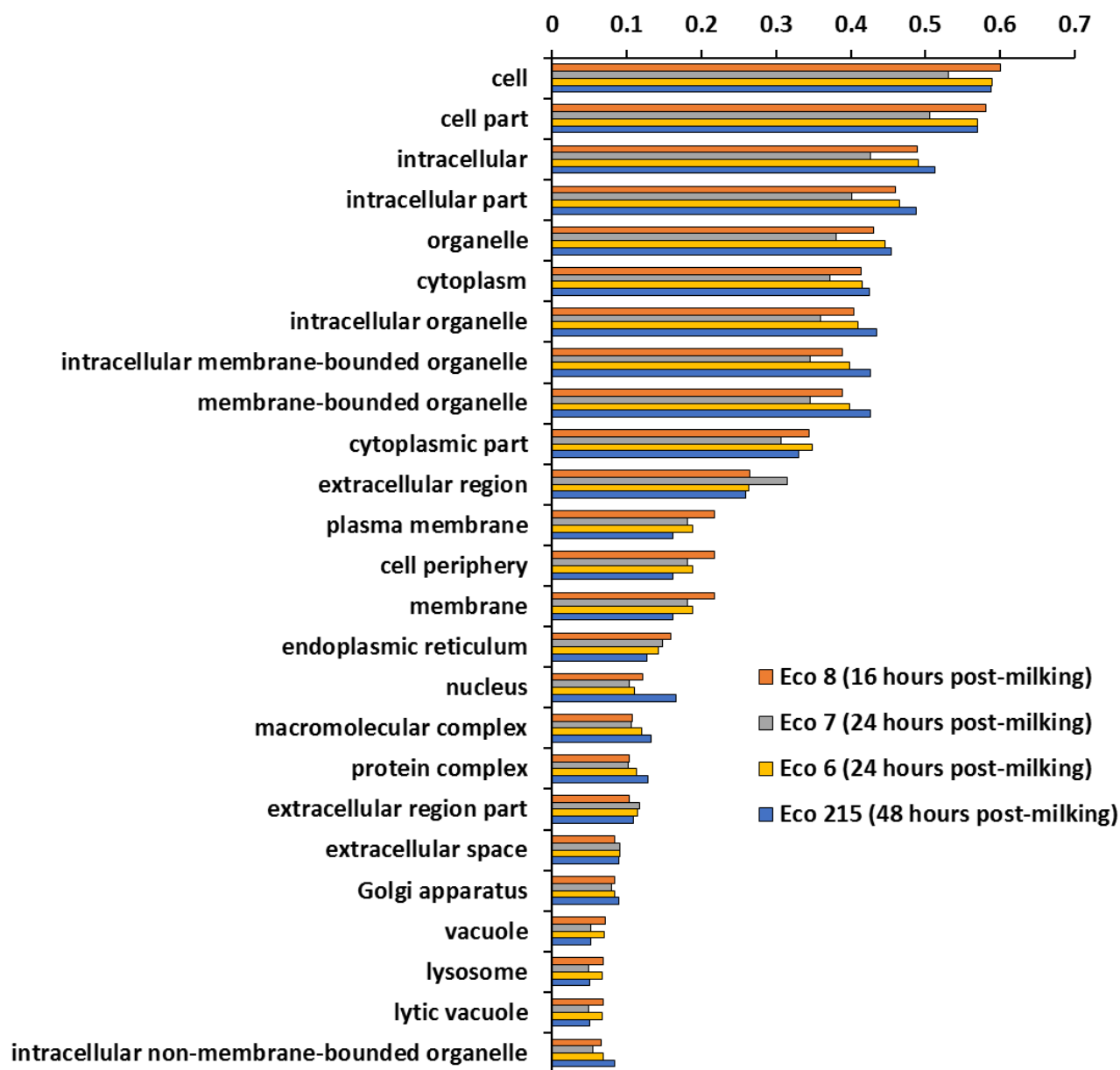
Supplementary figure S12. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Cellular component' terms for transcripts which are unique to each individual timepoint following milking in the venom gland secretome of the painted saw-scaled viper (*Echis coloratus*).



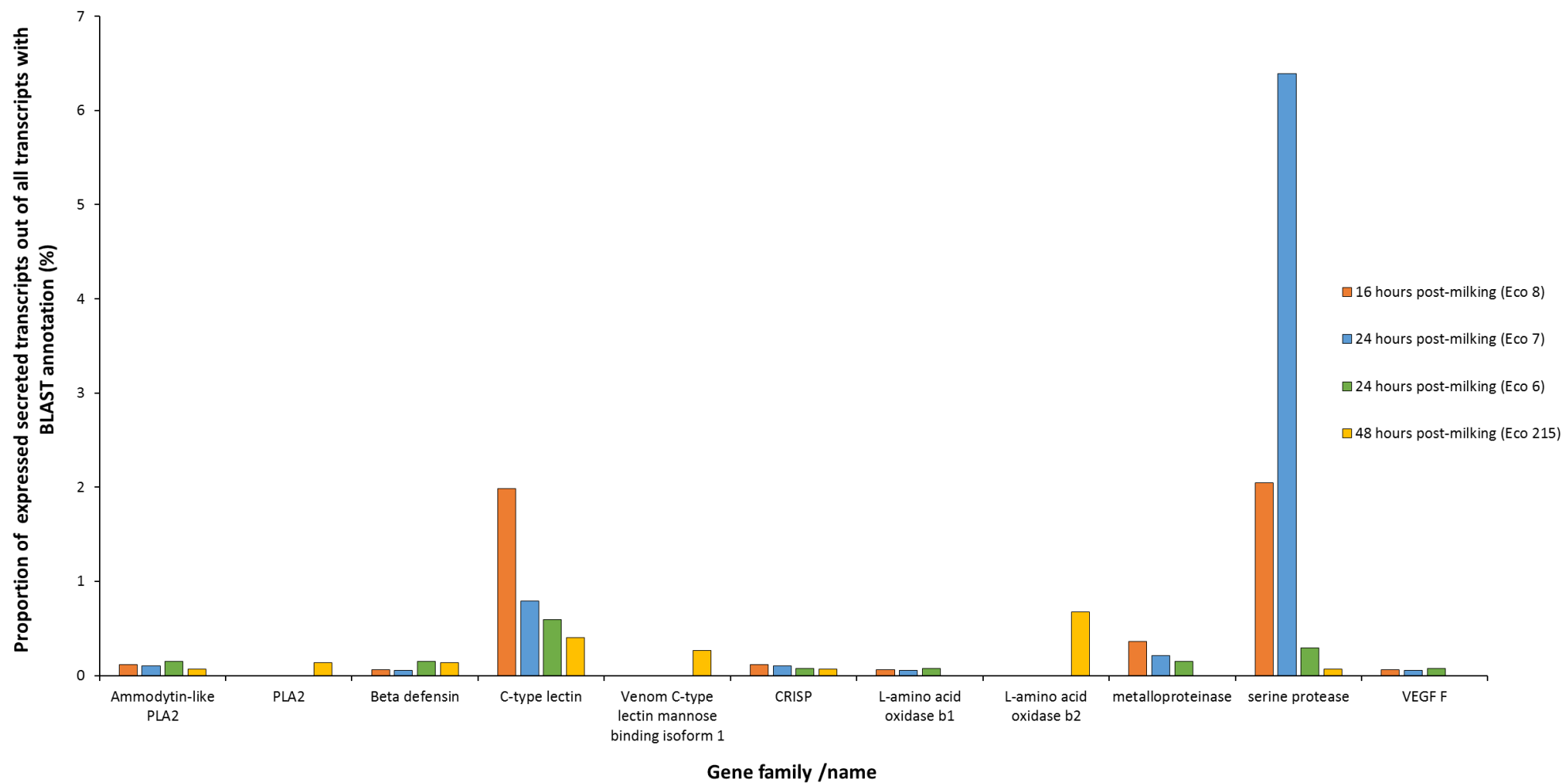
Supplementary figure S13. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Biological process' terms for painted saw-scaled viper (*Echis coloratus*) venom gland secretomes taken at different timepoints post-milking



Supplementary figure S14. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Molecular function' terms for painted saw-scaled viper (*Echis coloratus*) venom gland secretomes taken at different timepoints post-milking



Supplementary figure S15. Proportion of transcripts assigned to each of the top 25 gene ontology (GO) slim 'Cellular component' terms for painted saw-scaled viper (*Echis coloratus*) venom gland secretomes taken at different timepoints post-milking



Supplementary figure S16. Proportion of secreted transcripts belonging to toxin families expressed in the venom gland of *Echis coloratus* at different timepoints post-milking